Extenuation of Fish Visceral Waste as a Component Substitute of Microbiological Media

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Research Article

Abstract

Fishes and fishery by-products are a good source of proteins, high-quality amino acids, fatty acids, and minerals. Fishes are next only to meat and poultry as a staple food of most of the countries. Aquaculture has thus become an important activity in many countries which contributes to 25% of the total world seafood supply (Srinivasan and Saranraj, 2017). The World Bank Group Agriculture action plan highlights the expected projection in global fish supply (1,86,842 MT) and consumption (1,51,771 MT) by 2030. The remaining will be wasted. The dumping of this waste into the sea is a very unscientific practice as it undergoes decomposition and generates toxic by-products. This waste can be converted into a more functional form, hydrolysate, which possesses a high nitrogen content (Wangkheirakpam et al., 2019). The present study focused on finding the potential of fish protein hydrolysate (FPH) as a low-cost alternative of peptone in microbial culture media. The efficiency of fish protein hydrolysate (Catla catla and Labeo rohita) as a peptone source for bacteria (pathogenic and non-pathogenic strains) was compared with commercial peptone. The growth of bacterial strains was found better in FPH as compared to that in commercial media. The growth of Acinetobacter baumanii A87 was obtained 17% higher in formulated media as compared to that in commercial media. Similarly, among the pathogenic strains, Salmonella bongori BR1859 exhibited 10% higher growth in formulated media. Conversion of fish visceral waste into protein hydrolysate is thus an environment-friendly approach and also produces reasonable raw material for microbial media formulation (Parvathy et al., 2018). Such an application of fish visceral waste has been stated earlier; however, a comparison of both pathogenic and non-pathogenic strains together has not been reported yet.

Keywords: Fish visceral waste, fish protein hydrolysate, microbiological media, peptone, microbial growth, microbial strain

1. Introduction

Fishes are next only to meat and poultry as a staple food of most of the countries. Aquaculture has thus become an important activity in many countries which contributes to 25% of the total world seafood supply (Srinivasan and Saranraj, 2017). By the year 2025, the generation of solid waste is expected to reach 19 billion tones (Geethanjali and Subash, 2013). The fish waste generates at a huge scale due to annual enhancement in fish production. According to a World Bank report, by 2030 the global fish supply is estimated to increase by 5 lac metric tons. Of this, only 4 lac metric tons will be consumed, and the remaining will be wasted. This waste is usually dumped into water bodies which is a very unscientific practice as it deteriorates the water quality and structure of the planktonic community (Alajil, 2015).

However, animal feed and manure can be derived from this waste (Swanepoel and Goosen, 2018) but they possess low economic value. Moreover, fish visceral waste is rich in proteins, essential fatty acids, vitamins, antioxidants, beneficial amino acids and peptides, minerals, and trace metals (Rajeswari *et al.,* 2018). The exploitation of this waste as a substituent of peptone in microbiological growth media would lead to the valorization of fish visceral waste (Parvathy *et al.,* 2018).

Microbial growth media provides nutrients for the growth and maintenance of micro-organisms. Of all the components of media, peptone is the most expensive as microbes require nitrogen for building proteins and nucleic acids. At present, the commercial peptones appear from the bovine or porcine origin, and also from plants and yeasts. Due to recent outbreaks of bovine spongiform encephalopathy, zoonosis, and religious constraints (Silvipriya *et al.,* 2015), peptones of non-meat origin are becoming increasingly important (Fallah, 2015). Biotechnological fermentation industries and routine microbiological experiments (Berde and Berde, 2015) have raised the demand for microbial growth media and an inexpensive peptone source (Safari *et al.,* 2012). Thus, the availability of low-cost media is the need of the day. Apart from having a good nitrogen content, fish trash does not coagulate in hot water (Duffose *et al.,* 2001), which further supports microbial growth. Scientists (Soltanmoradi and Hedayatifard, 2015) have reported growth of the bacteria on fish peptone.

This study was undertaken to assess the suitability of fish protein hydrolysate for preparing low-cost media for different kinds of bacteria- pathogenic, and non-pathogenic. For this, three microbiological media were used: (1) commercial media, (2) peptone-free media and, (3) fish peptone media. As a criterion for comparison, the growth of selected strains was observed in terms of optical density at 595nm as growth yield.

2. Materials and methods

2.1 Collection of samples

Fish waste was bought from the local fish market (Shahpura, Bhopal) in an icebox and transferred to Molecular Biology Laboratory, Department of Biotechnology, Barkatullah University, Bhopal within 30 min.

2.2 Collection of Bacterial cultures

The cultures employed for this study have been summarized in Table1 and were obtained from the Department of Biotechnology, Barkatullah University, Bhopal.

2.3 Preparation of fish peptone medium

10g fish viscera were washed thoroughly with cold distilled water and mixed with distilled water (1:10). To this extract, 3M NaOH was added in the ratio 1:10. The hydrolysis process was done in an incubator shaker at 150 rpm at 50°C for 1.5 hr. After this, the solution was centrifuged at 5000 rpm, 4°C for 15 minutes (Husin *et al.* 2015). The supernatant was collected and protein content was determined Lowry's method (Lowry *et al.,* 1951) using bovine serum albumin (BSA) as standard. Fish peptone medium was prepared using 0.5% of fish protein hydrolysate in place of a nitrogen source.

2.3 Microbial Growth

Both pathogenic and non-pathogenic strains were used to test the microbial growth performance on fish protein hydrolysate. Strains were grown at 37 °C in 50ml of liquid media in triplicates. Cultivation was performed for one week on a rotary shaker. The growths of both microbes were monitored every 12 hours by measuring optical density (595nm) (Kurbanoglu and Kurbanoglu, 2002).

3. Results and Discussions

The growth curves of bacterial strains on different media are shown (Fig1 and Fig2). The data were analyzed using analysis of variance (ANOVA) and values significant above confidence level 95% ($p<0.05$) were accepted. The error bars in the graph indicate the significant difference.

As evident from the curves, the growth of all the selected pathogenic and non-pathogenic strains in fish peptone media surpassed that in commercial media. All the strains exhibited a log phase up to 48hrs. Growth in peptone-free media was least in all the cases as nitrogen is an essential component required for microbial growth (Soltanmoradi and Hedayatifard, 2015). Fish-based peptone has also been shown to perform better than commercial nitrogen sources by Deraz *et al.,* (2011) and Fallah *et al.,* (2015).

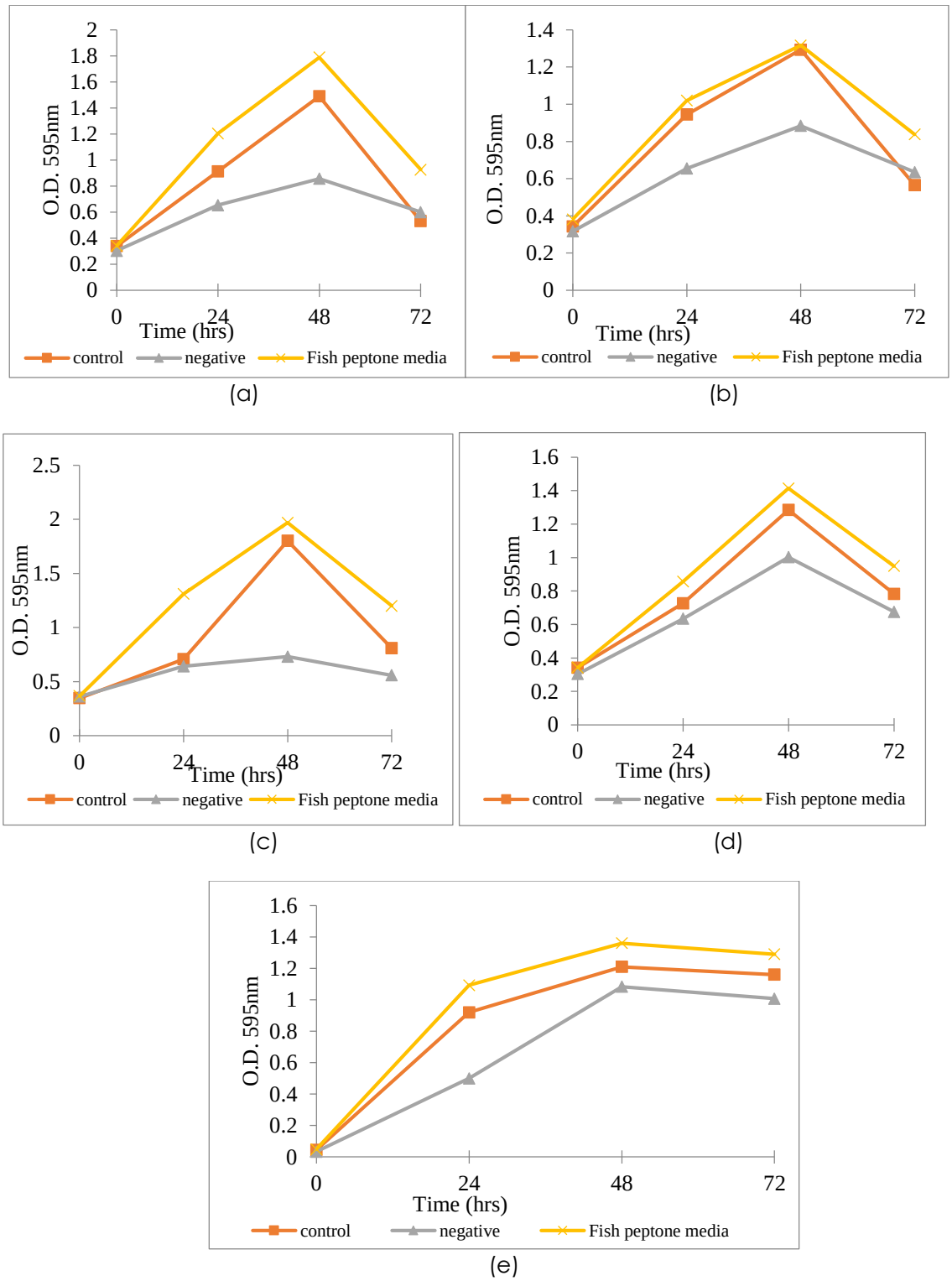

Fig. 1: Growth pattern of Non-Pathogenic strains on commercial media, negative control and fish peptone medium where, (a)*Acinetobacter baumanii* A87 (b) *Staphylococcus aureus* WT34 (c) *Pseudomonas fluorescence* W45 (d) *Escherichia coli* S09 and (e) *Bacillus pumilus* SAPR032

The best growth yield among non-pathogenic strains was observed in *Acinetobacter baumanii* A87 (O.D. 1.97). Similarly, among the pathogenic strains, *Salmonella bongori BR1859* exhibited maximum growth at O.D. 1.67. The strains also showed the highest difference of growth upon formulating the media. These results were in line with the findings of Beaulieu *et al.,* (2009) and Taskin *et al.,* (2016) who found better growth of genera of *Pseudomonas* and, *Bacillus*, *Staphylococcus,* and *Escherichia* in fish peptone as

nitrogen source. In a recent study conducted by Jaziri *et al.,* (2020) *Escherichia* coli and *Staphylococcus aureus* were found to exhibit excellent growth in fish protein hydrolysate as a peptone source due to good (Gly, Gln, Pro, and Ala) amino acid content. However, these results were in contrast with the findings of Krishnaswamy and Lahiri (1963) who monitored the growth of genera *Escherichia* and *Bacillus* in fish peptone media and commercial media and reported better growth in the latter. The selection of species and the procedure followed may be the reason of such contradiction.

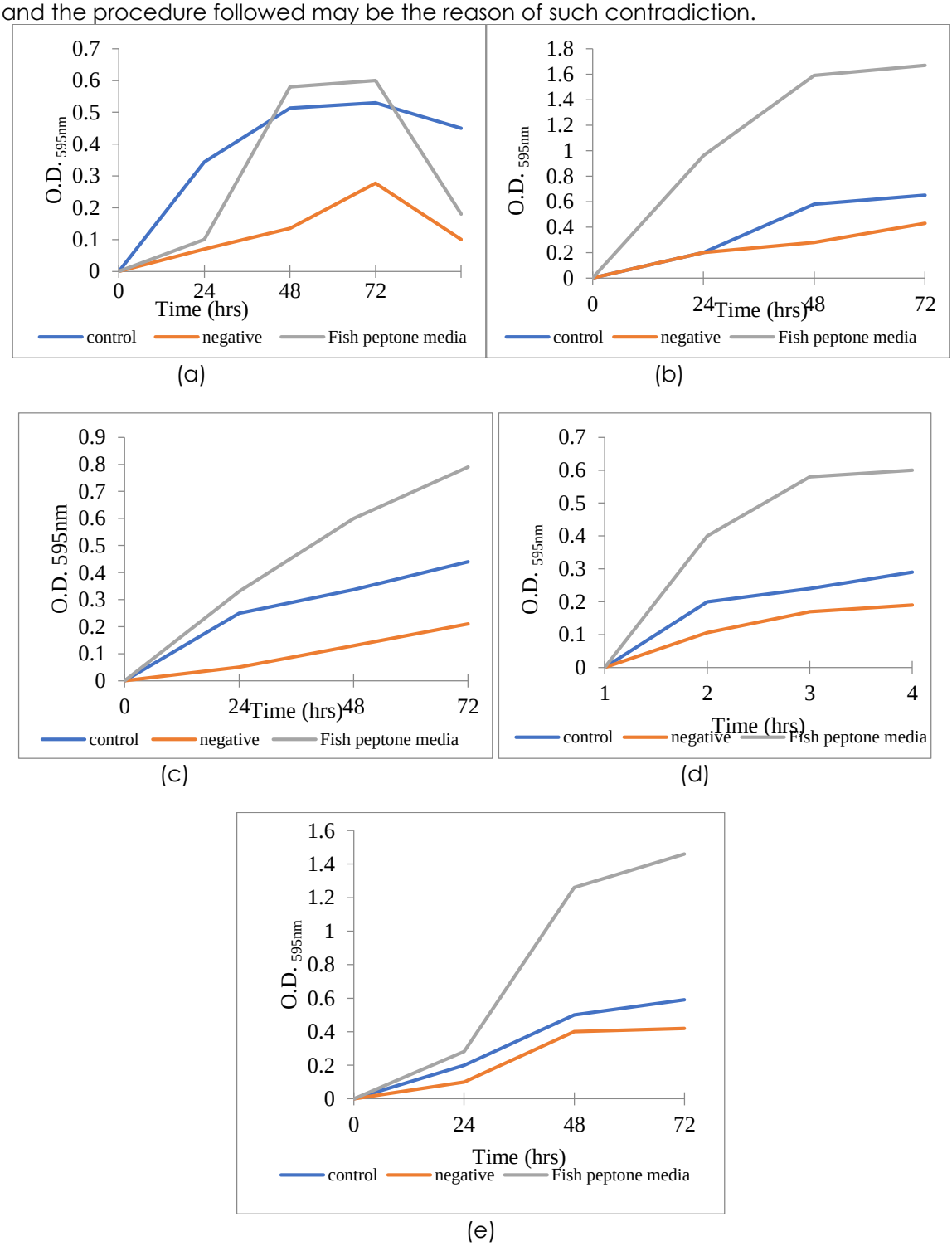

Fig. 2. Growth pattern of Pathogenic strains on commercial media, negative control and fish peptone medium where, A. *Comamonas testosterone* KS0043 B. *Salmonella bongori* BR1859 C. *Klebsiella oxytoca* ATCC13182 D. *Bordetella hinzii* OLMG13501 and E. *Morganella morgaini* M11 using *Catla* and *Labeo* visceral waste as peptone substitute

Since the growth of *S. aureus* in fish-derived peptone requires the presence of specific amino acids such as Leu, Arg, Val, Met, Cys, and His (Taskin *et al.,* 2016). The results indicate the presence of the above amino acids in the hydrolysate.

Table 1: Pathogenic and Non-Pathogenic bacterial strains chosen for this study

S.No.	Non-pathogenic	Pathogenic
1.	*Acinetobacter baumanii* A87	*Commamonas testosterone* KS0043
2.	*Staphylococcus aureus* WT34	*Salmonella bongori* BR1859
3.	*Pseudomonas fluorescence* W45	*Klebsiella oxytoca* ATCC13182
4.	*Escherichia coli* S09	*Bordetella hinzii* OLMG13501
5.	*Bacillus pumilus* SAPR032	*Morganella morgaini* M11

Table 2: Composition of culture medium used for microbial growth

S.No.	Ingredients	Commercial media	Peptone-free media	Fish peptone medium
1.	Beef extract	3g	3g	3g
2.	NaCl	5g	5g	5g
3.	Peptone	5g	0	1ml
4.	Distilled water	1000ml	1000ml	1000ml
	pH	6.9	6.9	6.9

4. Conclusions

The present study revealed that easily accessible fish waste can be a cost-efficient source of nitrogen over commercial peptone for culturing a diverse group of microbes (pathogenic and non-pathogenic). The fish peptone obtained upon hydrolyzing fish visceral waste has proved as an alternative to the exorbitant component of microbial media. Such an application not only delivers innovation by adding value to fisheries waste but also increases energy conservation and recycling consciousness. This can reduce the cost of large-scale production for biotechnological and clinical applications. However, sustainability and stability of hydrolysis for industrial-scale production is required as a practical approach because partial deamination of some amino acids may affect the results.

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Author Contributions: Prof. Ragini Gothalwal conceived the idea and Dr. Charu Batav collected the data; Prof. Ragini Gothalwal and Dr. Charu Batav analyzed the data; and Dr. Charu Batav wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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